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Overview of High Fluorescence Ratio (HFR), Middle Fluorescence Ratio (MFR), Mean Cell Volume (MCV), and Mean Cell Hemoglobin (MCH) in Patients with Hemoglobin Levels Below 8

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ABSTRACT

Background & Objective: Anemia occurs when a person has a deficiency of red blood cells or hemoglobin, with the threshold for anemia defined as hemoglobin levels below 12 g/dl. This condition is commonly found in medical practice, especially anemia caused by iron deficiency, vitamin B12 deficiency, folate deficiency, chronic disease, or bleeding. This study aimed to describe the high fluorescence ratio (HFR), middle fluorescence ratio (MFR), mean cell volume (MCV), and mean cell hemoglobin (MCH) in patients with hemoglobin levels below 8. **Method:** This was a descriptive study using purposive sampling, with a total of 30 respondents. **Result:** The results showed that 9 patients (30%) had high HFR and MFR with normal MCV and MCH; 6 patients (20%) had normal HFR and MFR with normal MCV and MCH; 5 patients (17%) had high HFR and MFR with low MCV and MCH; 3 patients (10%) had high HFR, normal MFR, and low MCV and MCH; 2 patients (7%) had high HFR and MFR with normal MCV and low MCH; 2 patients (7%) had normal HFR and MFR with low MCV and normal MCH; 1 patient (3%) had normal HFR and MFR with low MCV and MCH; 1 patient (3%) had normal HFR, high MFR, with low MCV and normal MCH; and 1 patient (3%) had normal HFR, low MFR, and normal MCV and MCH. **Conclusion:** This study indicates that most patients with severe anemia (Hb < 8) showed a relatively good regenerative response (as indicated by high HFR and MFR), although variations in anemia types were also observed based on MCV and MCH values, reflecting possible different causes and classifications of anemia.

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Introduction

Anemia is a condition in which the number of red blood cells, hematocrit value/volume, and red blood cell indices decrease. Anemia occurs when red blood cells are either too small or too large, and when hemoglobin levels are below 12 g/dL, the individual is considered anemic. Several factors may cause anemia, including folic acid deficiency, vitamin B12 deficiency, iron deficiency, chronic disease, and bleeding (Atika, 2011).

According to the World Health Organization (WHO), anemia globally affects 1.62 billion people, equivalent to 24.8% of the population. Data from Riskesdas 2013 reported that 37.1% of adolescent girls suffered from anemia, which increased to 48.9% in Riskesdas 2018, with the highest proportion found among individuals aged 15–24 years and 25–34 years (Nasruddin et al., 2021).

Anemia is a condition in which the body lacks red blood cells or has low hemoglobin levels, leading to a reduced capacity of the blood to transport oxygen throughout the body. Various laboratory examinations can be performed to detect and evaluate anemia, including High Fluorescence Ratio (HFR), Middle Fluorescence Ratio (MFR), Mean Cell Volume (MCV), and Mean Cell Hemoglobin (MCH). HFR and MFR examinations help assess bone marrow activity in producing red blood cells, while MCV and MCH provide information about the size and hemoglobin content of red blood cells, which are essential in determining the type of anemia a patient is experiencing (Lfr mfr hfr, n.d.; Suburwibowogmailcom, 2024).

High Fluorescence Ratio (HFR) refers to a group of reticulocytes that emit stronger fluorescence due to their abundant RNA content. This indicates that the cells are relatively newly formed and still in the early stages of maturation. This test can be performed using the flow cytometry method, where HFR measures the fluorescence ratio of reticulocytes in the final stage of maturation (Robert B, 2023; Obstfeld et al., 2024; Laboratory of Physiology, Structure, and Animal Development, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, 2019).

Middle Fluorescence Ratio (MFR) refers to reticulocytes (immature red blood cells) that show moderate fluorescence levels when analyzed using flow cytometry. In principle, flow cytometry measures the fluorescence ratio of reticulocytes at the intermediate stage of maturation. MFR is used to assess bone marrow function in producing red blood cells (Robert B, 2023; Obstfeld et al., 2024; Laboratory of Physiology, Structure, and Animal Development, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, 2019).

Mean Cell Volume (MCV) is the average volume of red blood cells. A low MCV value indicates microcytic anemia, while a high MCV value indicates macrocytic anemia. This examination can be conducted using flow cytometry, which involves measuring the volume of individual red blood cells with the aid of a laser beam. Each red blood cell passes through the laser, and the scattered light is analyzed by a detection system to determine the average size of the cells (Eva Ayu, 2023; Laboratory

of Physiology, Structure, and Animal Development, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, 2019).

Mean Cell Hemoglobin (MCH) represents the amount of hemoglobin per red blood cell, which reflects the weight of hemoglobin contained within each red blood cell. A decrease in MCH is observed in microcytic and hypochromic anemia. This test uses flow cytometry, where red blood cells are lysed to release the hemoglobin they contain. A special reagent is then used to form a fluorescent complex with hemoglobin. As this complex passes through the laser beam in the flow cytometer, the fluorescence intensity emitted is proportional to the hemoglobin content in the sample (Eva Ayu, 2023; Laboratory of Physiology, Structure, and Animal Development, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, 2019).

Based on the background described above, the researcher is interested in conducting a study entitled "An Overview of High Fluorescence Ratio (HFR), Middle Fluorescence Ratio (MFR), Mean Cell Volume (MCV), and Mean Cell Hemoglobin (MCH) in Patients with Hemoglobin Levels Below 8", as this type of study has rarely been conducted.

Objective

This study aimed to describe the High Fluorescence Ratio (HFR), Middle Fluorescence Ratio (MFR), Mean Cell Volume (MCV), and Mean Cell Hemoglobin (MCH) in patients with hemoglobin levels below 8.

Method

This research employed a descriptive study design to determine the profile of HFR, MFR, MCV, and MCH in patients with hemoglobin levels below 8. The study was conducted from March to May 2025. The study population consisted of 44 patients with hemoglobin levels below 8 at QIM Hospital, Batang Regency. With a margin of error of 0.1 (10%), a sample size of 30 patients was obtained using Slovin's formula, selected through purposive sampling. Data analysis was carried out descriptively, with the results presented in tabular form.

Results

The study was conducted in March 2025 with a total of 30 samples. After analyzing the High Fluorescence Ratio (HFR), Middle Fluorescence Ratio (MFR), Mean Cell Volume (MCV), and Mean Cell Hemoglobin (MCH) in patients with hemoglobin levels below 8, the results obtained are presented in Table 1 as follows:

TABLE 1. Results of HFR, MFR, and MCV, MCH Examination in Patients with Hemoglobin Levels Below 8

Sample Code	Hemoglobin g/dl	HFR	MFR	MCV	MCH
S1	7,7	0,0	5,0	84,6	28,9
S2	6,8	3,5	16,0	61,0	17,3
S3	7,5	0,4	9,8	78,7	26,2

Sample Code	Hemoglobin g/dl	HFR	MFR	MCV	MCH
S4	2,4	5,5	14,3	90,2	29,3
S5	6,5	11,8	8,6	79,6	24,1
S6	7,8	5,6	16,8	71,1	20,3
S7	6,8	0,0	4,9	78,7	27,3
S8	2,5	15,8	26,2	66,9	20,2
S9	6,8	1,5	7,2	81,1	27,3
S10	7,0	0,0	5,3	96,0	31,3
S11	7,7	0,9	10,7	75,3	27,2
S12	7,8	8,9	16,4	80,9	25,7
S13	7,3	1,7	13,9	77,0	25,4
S14	4,8	13,1	18,7	92,9	31,0
S15	6,8	15,5	19,7	85,9	25,3
S16	4,9	6,8	22,7	94,4	30,6
S17	7,8	16,1	21,5	85,7	29,9
S18	6,1	0,5	5,8	81,3	27,2
S19	6,8	0,6	6,1	81,2	27,2
S20	5,8	13,9	17,5	75,0	22,3
S21	6,7	6,4	11,2	64,7	20,1
S22	7,4	2,0	7,4	66,0	21,3
S23	6,7	0,4	0,8	86,1	28,3
S24	6,8	0,0	5,0	85,5	28,2
S25	7,5	2,2	16,2	81,8	27,4
S26	5,2	14,2	15,3	89,7	29,7
S27	7,9	16,4	19,1	89,3	27,0
S28	5,3	0,9	8,9	78,4	27,3
S29	6,8	6,4	10,1	64,7	20,5
S30	7,4	4,1	15,3	88,0	27,8

TABLE 2. Percentage of MFR, HFR, and MCV, MCH in Anemia Patients with Hemoglobin Levels Below 8

No	HFR	MFR	MCV	MCH	Total	Presentage
1	High	High	Normal	Normal	9	30%
2	Normal	Normal	Normal	Normal	6	20%
3	High	High	Low	Low	5	17%
4	High	Normal	Low	Low	3	10%
5	High	High	Normal	Low	2	7%
6	Normal	Normal	Low	Normal	2	7%
7	Normal	Normal	Low	Low	1	3%
8	Normal	High	Low	Normal	1	3%
9	Normal	Low	Normal	Normal	1	3%
Total					**30**	**100%**

Discussion

Based on Table 4.2, the percentage results of HFR, MFR, MCV, and MCH examination in 30 anemia patients with hemoglobin levels below 8 were as follows: high HFR and MFR with normal MCV and MCH in 30%; normal HFR and MFR with normal MCV and MCH in 20%; high HFR and MFR with low MCV and MCH in 17%; high HFR with normal MFR and low MCV and MCH in 10%; high HFR and MFR with

normal MCV but low MCH in 7%; normal HFR and MFR with low MCV and normal MCH in 7%; normal HFR and MFR with low MCV and MCH in 3%; normal HFR with high MFR and low MCV but normal MCH in 3%; and normal HFR with low MFR and normal MCV and MCH in 3%.

In this study, severe anemia (Hb < 8 g/dL) with high HFR and MFR but normal MCV and MCH (30%) reflected regenerative normocytic normochromic anemia. This indicates that although red blood cells are normal in size and hemoglobin content, the bone marrow responds to anemia by releasing more young reticulocytes into circulation. Such a pattern is commonly seen in hemolysis or acute bleeding, where red cell loss exceeds production (Robert B, 2023).

Cases with normal HFR and MFR but normal MCV and MCH (20%) suggested non-regenerative normocytic normochromic anemia, meaning that red cells appear normal, but the bone marrow has not increased reticulocyte output—pointing to impaired erythropoiesis rather than excessive loss or destruction of red blood cells (Robert B, 2023).

High HFR and MFR with low MCV and MCH (17%) reflected regenerative microcytic hypochromic anemia, characterized by small and pale red cells, with the bone marrow compensating by increasing the release of young reticulocytes (Robert B, 2023).

High HFR with normal MFR and low MCV and MCH (10%) indicated microcytic hypochromic anemia with partial regenerative response, where the bone marrow releases the youngest reticulocytes, but mid-maturing reticulocytes remain limited, suggesting that erythropoiesis is stimulated but not yet optimal (Robert B, 2023).

High HFR and MFR with normal MCV but low MCH (7%) described regenerative normocytic hypochromic anemia. Here, red cells are normal in size but contain less hemoglobin per cell, while the bone marrow compensates by releasing more young reticulocytes (Robert B, 2023).

Normal HFR and MFR with low MCV but normal MCH (7%) indicated non-regenerative microcytic normochromic anemia, meaning the red cells are small but hemoglobin per cell is preserved, with bone marrow not increasing reticulocyte production, suggesting defective erythropoiesis (Robert B, 2023).

Normal HFR and MFR with low MCV and MCH (3%) represented non-regenerative microcytic hypochromic anemia, where red cells are small and pale, and the bone marrow fails to respond by producing more reticulocytes, indicating impaired production rather than increased loss (Robert B, 2023).

Normal HFR with high MFR and low MCV but normal MCH (3%) suggested partially regenerative microcytic normochromic anemia. This means the red cells are small but still maintain sufficient hemoglobin per cell, and the bone marrow has started to release reticulocytes, although not optimally. Such a pattern is often found in early iron deficiency, mild acute bleeding, or hemolysis with limited erythropoietic stimulation (Robert B, 2023).

Conclusion

Most patients with severe anemia (Hb < 8 g/dL) demonstrated a good regenerative response, indicated by elevated HFR and MFR. Variations in MCV and MCH values reflected different anemia types, suggesting diverse underlying causes. Future research is recommended to include larger sample sizes and additional hematological parameters, such as complete blood count indices and reticulocyte production index, to provide a more comprehensive understanding of anemia profiles. It is also suggested to investigate the underlying causes of anemia through biochemical and clinical assessments, so that diagnostic accuracy and patient management can be improved.

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